S1.P24

Subunit F of A-ATP synthases is an ATPase stimulating subunit

Dhirendra Singh[a], Shashi Bhushan[b], Gerhard Grüber[a], Hendrik Sielaff[a]
[a]*School of Biological Sciences, Nanyang Technological University, Singapore*
[b]*Division of Structural Biology and Biochemistry, School of Biological Sciences, Nanyang Technological University, Singapore*
E-mail address: dhirendr001@e.ntu.edu.sg

Archaeal A-ATP synthases are composed of 9 different subunits in the proposed stoichiometry of A_3:B_3:C:D:F:$(EH)_2$:*a*:*c*, where the soluble domain A_1 has a hexameric head made up of A_3:B_3. Subunits C, D, and F form the central stalk, and a heterodimer of subunits E and H forms the peripheral stalk [1]. The membrane embedded integral A_O domain contains the subunits *a* and *c*, which are responsible for ion-translocation. Genes encoding the subunits A–F have been synthesized to express the complexes A_3B_3D and A_3B_3DF of the chemically-driven motor A-ATP synthase from *Methanosarcina mazei* Gö1. A new purification protocol has been developed to generate highly pure proteins, which were used for 2D-classification and 3D-reconstructions of negatively stained EM-images. The 3D-reconstruction of the A_3B_3D- and A_3B_3DF-complexes enabled the assignment of the subunits in the complex. ATP hydrolysis activity of the purified proteins showed that the A_3B_3DF-complex is seven times more active than A_3B_3D, indicating that subunit F acts as a stimulator of hydrolysis activity in the A_1-ATPase. Mutational studies have been used to map the critical epitopes in the coupling subunit F.

Reference

[1] G. Grüber, M.S.S. Manimekalai, F. Mayer, V. Müller, ATP synthases from archaea: the beauty of a molecular motor, Biochim Biophys Acta-Bioenergetics, 1837 (2014), 940–952.

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S1.P25

Single-molecule approach to compare the rotation of F_1-ATPase with the archaeal A_1-ATPase

Hendrik Sielaff, Dhirendra Singh, Lavanya Sundaraman, Ardina Grüber, Gerhard Grüber
School of Biological Sciences, Nanyang Technological University, Singapore
E-mail address: hsielaff@ntu.edu.sg

The rotary ATP synthase is a unique molecular machine consisting of two rotary nanomotors, which work as a generator/motor and can synthesize ATP by utilizing an electrochemical potential of H^+/Na^+ over the membrane, or vice versa. The soluble complex that is capable of hydrolyzing ATP only consists of subunits $\alpha_3\beta_3\gamma$ in the bacterial F-type ATPase (F_1), or of subunits A_3B_3DF in the archaeal A-type ATPase (A_1). For the bacterial F_1-ATPase a single-molecule rotation assay was developed to visualize the rotational movement of the rotary γ subunit within the static hexagonal barrel of $\alpha_3\beta_3$. Subunits $\alpha_3\beta_3$ were attached via His-tags to a cover slide while a fluorescent probe was attached to subunit γ. Upon addition of ATP the rotational movement of subunit γ was monitored via the fluorescent probe by using TIRF-microscopy. Movies were recorded by a high speed CMOS-camera. Here, for the first time, we applied this technique to the archaeal system using the A_3B_3DF complex of the A-ATP synthase from *Methanosarcina mazei* Gö1. The A_3B_3DF complex was immobilized via subunits A_3B_3, while the fluorescent probe was coupled to subunit D, enabling the comparison of the movements of the molecular motors F- and A-ATP synthase.

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Subunit ε of *Mycobacterium tuberculosis* F-ATP synthases is critical in ATPase inhibition

Lavanya Sundararaman, Sandip Basak, Gerhard Gruber
School of Biological Sciences, Nanyang Technological University, Singapore
E-mail address: lavanya2@e.ntu.edu.sg

Functional F-ATP synthase is critical for the viability of pathogenic bacteria such as *Mycobacterium tuberculosis*. The mycobacterial F_1F_O ATP synthase consists of the ATP synthesizing F_1 section ($\alpha_3\beta_3\gamma\delta\varepsilon$) and the proton-translocating F_O part (subunits $a_1b_2c_{9-15}$). Recently we have employed a complementary approach of solution X-ray scattering and NMR spectroscopy to determine the solution structures of *M. tuberculosis* subunit ε and its C-terminal domain, respectively [1]. Based on the physiological role of ε regulation in the ATP synthase, bacteria show variations in length and composition of the amino acid sequence of this C-terminus. Here we used mutational studies to map the critical epitopes and residues of the coupling and regulatory subunit ε from *M. tuberculosis* F-ATP synthase.

Reference

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S1.P27

Tmem70 gene knockout alters biogenesis of ATP synthase and leads to embryonal lethality in mice

Marek Vrbacky[a], Jana Kovalcikova[a], Hana Nuskova[a], Kallayanee Chawengsaksophak[b], Inken Beck[b], Radislav Sedlacek[b], Pavel Hozak[b], David Sedmera[a], Josef Houstek[a]
[a]*Institute of Physiology ASCR, v.v.i., Czech Republic*
[b]*Institute of Molecular Genetics ASCR, v.v.i., Czech Republic*
E-mail address: vrbacky@biomed.cas.cz

Tmem70 is a 21 kDa transmembrane protein localized in the inner mitochondrial membrane and involved in the biogenesis of the eukaryotic ATP synthase. Tmem70 mutations are responsible for isolated deficiency of ATP synthase resulting in a severe, often fatal neonatal mitochondrial encephalocardiomyopathy in patients. To better understand the function of this ancillary biogenetic factor, we generated Tmem70 knockout mice by embryonic stem cell technology and verified the lack of the Tmem70 expression in the homozygous embryos by quantitative RT-PCR. While the heterozygous mice were viable, the homozygous embryos revealed profound growth retardation and died at the stage of approximately 8.5–9.5 days post coitum. More detailed morphological analysis indicated the disturbance of cardiovascular system. Blue native electrophoresis demonstrated isolated decrease of ATP synthase complex in homozygous embryos similarly to the samples of fibroblasts from human patients. The content of fully assembled F1Fo ATP synthase detected by WB was decreased in homozygous embryos to less than 25% compared to that in wild type embryos, while the F1 subcomplex of ATP synthase in the homozygous embryos increased. Similarly, ATPase in-gel hydrolytic activity revealed identical changes. In addition, transmission electron microscopy showed disturbed mitochondrial crista morphology in Tmem70 knockout embryos compared to wild type embryos. Our results thus demonstrate that Tmem70 ablation in the mouse model has lethal